# EFFECTIVENESS OF MENIRAN (Phyllanth us nir uri Linn ) AS ANTIBACTERIAL FOR ANTIBIOTICS RESISTANCE ENTEROTOXIGENIC Escherichia coli

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Research Report

# EFFECTIVENESS OF MENIRAN (*PHYLLANTHUS NIRURI* LINN) AS ANTIBACTERIAL FOR ANTIBIOTICS RESISTANCE ENTEROTOXIGENIC *ESCHERICHIA COLI*

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***ABSTRACT***

*Escherichia coli (E. coli) can be isolated from the environment both inside and outside the hospes body. There were 89 serotypes in which showed 21% resistance to various antibiotics, such as enterotoxigenic E.coli. Alternative efforts are needed to overcome this, one of them through the use of medicinal plants, such as meniran (Phyllanthus niruri Linn). Meniran plant is an immunomodulator that serves to repair the immune system of the body. The aim of research is the research was done through several stages: isolation and identification of enterotoxigenic E. coli from several broiler farms in East Java using the polymerase chain reaction (PCR) method, E. coli resistance test against some antibiotics, making meniran extract and activation test against enterotoxigenic E. coli n The study was divided into five treatments: T0+ (group of chickens were infected by enterotoxigenic E. coli T0- (control group, not infected), T1 (infected by enteroxigenic E. coli + 20% meniran extract), T2 (infected by enterotoxigenic E. coli + 25% extract meniran), T3 (infected by enterotoxigenic E. coli + 30% extract meniran). Data were analyzed by ANOVA (Analysis of Variance). The results show that all of E. coli DNA isolates which tested by the PCR method show positive reactions at 600 bp. The next stage, the enterotoxigenic E. coli are resistant to some antibiotics, such as amoxicillin, ampicillin, erythromycin, cephalosporins, tetracycline, cloxacillin and gentamicin. Furthermore, the 30% Phyllanthus niruri Linn extract is effective as an antibacterial for antibiotic resistance enterotoxigenic E. coli. next necessary to write that: The 30% meniran extract is potential for kill of enterotoxigenic E. coli*

**Keywords:** *Phyllanthus niruri Linn, enterotoxigenic E. coli, antibiotic resistance, medicinal plants, immunomodulator*

***ABSTRAK***

*Escherichia coli (E. coli) dapat diisolasi dari lingkungan baik di dalam maupun di luar tubuh inang. Ditemukan 89 serotipe dimana 21% menunjukkan resistensi terhadap berbagai antibiotik, seperti E. coli enterotoxigenic. Diperlukan upaya alternative sebagai pengganti antibiotik, salah satunya melalui pemanfaatan tanaman obat, seperti meniran (Phyllanthus niruri Linn). Tanaman meniran merupakan imunomodulator yang berfungsi memperbaiki sistem imun tubuh. Penelitian ini melalui beberapa tahap yaitu: isolasi dan identifikasi E. coli enterotoxigenic dari beberapa peternakan ayam pedaging di Jawa Timur menggunakan metode polymerase chain reaction (PCR), uji resistensi E. coli terhadap beberapa antibiotik, pembuatan ekstrak meniran dan uji aktivasi meniran terhadap E. coli enterotoxigenic. Penelitian ini dibagi menjadi lima perlakuan: T0+ (kelompok ayam yang terinfeksi oleh E. coli enterotoxigenic), T0- (kelompok kontrol, ayam tidak terinfeksi enterotoksigenic E. coli, T1 (kelompok ayam yang terinfeksi oleh E. coli enterotoxigenic + ekstrak meniran dosis 20%), T2 (ayam terinfeksi oleh E. coli enterotoxigenic + ekstrak meniran dosis 25%), T3 (ayam terinfeksi E. coli enterotoxigenic + ekstrak meniran dosis 30%). Data dianalisis dengan ANOVA (Analisis Varians). Hasil penelitian menunjukan semua isolat DNA E. coli yang diuji melalui metode PCR menunjukkan reaksi positif berada pada posisi 600 bp. Pada tahap penelitian berikutnya ditemukan bahwa E coli enterotoksigenic resisten terhadap beberapa antibiotik, seperti: amoxicillin, ampicillin, eritromisin, cephalosporins, tetracycline, cloxacilin dan gentamicin. Selanjutnya 30% ekstrak meniran (Phyllanthus niruri Linn) 30% efektif sebagai*

*antibakteri untuk pencegahan resisten antibiotik dari E. coli enterotoxigenic. Kesimpulan dari penelitian ini, 30% meniran extract efektif unuk membunuh E. coli enterotoksigenic*

***Kata kunci:*** *Phyllanthus niruri Linn, E. coli enterotoxigenic, resisten antibiotik, tanaman obat, imunomodulator*

## INTRODUCTION

The poultry, especially chickens are farm animals which particularly vulnerable to diseases. This condition leads to decrease in productivity and causes major losses if the treatment is not successful.[1] One of the diseases that are common and detrimental to farmers is a bacterial infection such as *E. coli*.[2] *E. coli* especially due to enterotoxigenic from *E. coli* was commonly cause diarrheal disease in chickens, is called Colibacillosis.[3] The losses due to diseases such as colibacillosis is high chicken mortality which can reach 30%. Colibacillosis disease attacks young chickens until harvesting on the age around of 25-35 days old broiler and 40-50 days old layer.[4]

Incorrect diagnosis, treatment and control of *E. coli* infections often cause resistance to antibiotics.[5] There are 89 serotypes *E. coli* can be isolated from the host and 21% exhibit resistance to various antibiotics, because *E. coli* contains plasmids.[6]

Use of antibiotics should be re-evaluated, as well as against the enterotoxigenic and virulence factors of *E. coli*. The meniran (*Phyllanthus niruri* Linn) is a plant that can be used as an alternative prevention and treatment of diseases which caused by *E. coli*.[7] The chemicals contain in meniran are flavonoids and tannins.[8] The flavonoids action of *Phyllanthus niruri* Linn is an imunnomodulator whose role is to boost the immune system and improve dysfunctional of the immune system.[9] Immune dysfunctional is caused a decrease in number of immune cells such as T-CD5+,CD4+, CD8a+,CD8b+ in the lamina proria and intraepithelial villi intestine.[10]

The immune dysfunctional, which can lead to infection by bacterial or parasites with symptoms of chronic diarrhea and that, will increase mortality. From previous studies, the animal laboratory were conditioned immune dysfunctional in addition to obtain high expression of Hsp70, low expression of prostaglandin E2, expression of immunoglobulin A, histologically visible damage of intestinal mucosa epithelium and is showed villus atrophy, which will lead to decrease in the absorption function. The impact is as reduced nutritional intake and organs damage, including liver damage.[11] This will reduce the reproductive rate of both male[12] and female,[13,14] which in turn decreases productivity for livestock, especially poultry.

Tannins efficacious as an antibacterial (prevents bacterial growth) and hemostatic (bleeding stoped).[15] Phenolic compounds may be bactericidal or bacteriostatic depending on the concentration used. Antibacterial substances have various ways in inhibiting bacterial growth.[16] Damage to one of the structures of the bacterial cell can cause changes in the structure and action of bacteria. This can lead become stunted bacterial growth and even lead to cell death.[17] Cytoplasmic membrane is the outer part of the cytoplasm which is located under the cell wall, composed of proteins, lipids and carbohydrates. This membrane plays a role to regulate the incoming of matter such as water and mineral salts needed by the cell. The parts of the cell in the cytoplasm are ribosomes, nuclei, granules and mesosomes. Ribosomes are small follicles composed of proteins and ribonucleic acid (RNA), which their function were act in protein synthesis. The nucleus contains dioxyribonucleic acid (DNA) as a carrier of genetic information. Granules are chemical substances that can functionate as food reserves for cells. The mesosome is the fold of the cytoplasmic membrane into the cytoplasm. In connection with this, the damage to cell membranes by antibacterial substances can lead stunted cell growth and even result in the death of bacterial cells.[16]

The meniran plant as the composition of feed as well as a single oramixture of food ingredients, processed or unprocessed, which is given for animal's survival, production and breed.[18] The research purposes is isolation and identification of enterotoxigenic *E. coli* was antibiotic-resistant as the causative agent of loss and death of broilers. Furthermore, applying meniran (*Phyllanthus niruri* Linn) as antibacterial for the prevention enterotoxigenic *E. coli* of antibiotic-resistant in broiler.

## MATERIAL AND METHOD

The research was done through several stages:

**First stage.** Isolation and identification of *enterotoxigenic E. coli* from several broiler farms in East Java (Mojokerto and Tuban). The identification using the polymerase chain reaction (PCR) method[19] with primer: Forward (5'-TAGAGAAATTATCAAGTTAGTTCC-3') Reverse: (5'-ATAGTTATGAACATCTTGTTTAGC-3') (17).

**The second stage.** *E. coli* resistance test against some antibiotics were used dilution method, namely *Minimum Inhibitory Concentration* (MIC)[20] and *Minimum Bacteriocide Concentration* (MBC). The test were done through several antibiotics: amoxicillin, amphicillin, erythromycin, cephalosporins, tetracycline, cloxacilin and gentamicin.

**The third stage.** The synthesis of meniran extract with ethanol solven. Ried Meniran plants have been milled to obtain powder. Pollen meniran 1 kg extracted using maceration method by immersion in a solution of ethanol 96% as much as five liters for 3 x 24 hours. Stirring is

done twice, morning and afternoon. Maceration process is performed three times. The marinade filtrate is then filtered to evaporate using a rotary evaporator which will yield a concentrated plant extracts meniran[21] and then, meniran extract was already for application to broiler chicken.

**The fourth stage.** Activation test of the meniran extract against *enterotoxigenic E. coli* were applicated to 25 broiler chicken aged 19 days, with treatment as follow: *E. coli* infected T0+: broiler chickens infected with *E. coli* at 28 days of age with a concentration of $10^6$ CFU/ ml/chicken orally without meniran plant extract (control positive); T0-: broiler chickens at 28 days without any treatment (control negative); *E. coli* infected T1: broiler chickens 28 days of age with a concentration of $10^6$ CFU/ml/chicken orally and then given the extract of meniran plants with a dose of concentration of 20% /ml/chicken orally; *E. coli* infected T2: broiler chickens 28 days of age with a concentration of $10^6$ CFU/ml/chicken orally and then given the extract of meniran plants with a dose of concentration of 25%/ml/ chicken orally; *E. coli* infected T3: broiler chickens at 28 days of age with a concentration of $10^6$ CFU/ml/ chicken orally and they had been given the extract of meniran plants with a dose of concentration of 30%/ml/chicken orally.

Sampling for the calculation of the number of E. coli bacteria was done by killing the broiler chickens in each treatment to take 1 g of broiler chicken liver sample and inserted into a venoject tube containing 9 ml of physiological NaCl solution and labeled treatment for each sample. All samples that have been taken are saved into the coolbox.

Data on the number of *E. coli* with *Most Probable Number* (MPN) were analyzed by ANOVA (Analysis of Variance). The data was analysed using SPSS version 20. *p* value at the level of < 0,05 was refer to significant.

## RESULT AND DISCUSION

The isolation and identification of Escherichia coli bacteria results on broiler chicken liver samples from broiler farms in Tuban and Mojokerto, where showed of *E. coli* infected typical symptoms such as diarrhea and sepsis. The body was bluish, *E. coli* isolated bacteria was identified on hepar. 29 samples including 17 samples from Tuban and 12 samples from Mojokerto, were showed positive for *E.coli* in 20 samples consist of 12 samples from Tuban and 8 samples from Mojokerto. The results of *E.coli* isolation on EMBA media were showed metallic green colonies (Figure 1). Biochemical test results are as follows TSIA (+), Indole (+), Urea Agar (-) and SCA (-).

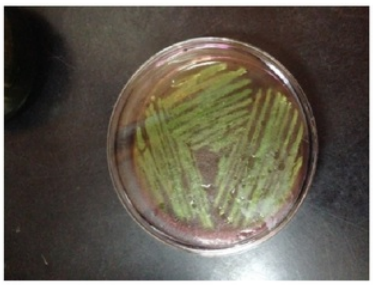

**Figure 1.** *E. coli* isolation in EMBA media.

The identification of *enterotoxigenic E. coli* DNA from several broiler farms in East Java (Mojokerto and Tuban) using the polymerase chain reaction (PCR) method was showed a positive reaction for all isolates tested the PCR fragments which was located position at 600 bp (Figure 2).

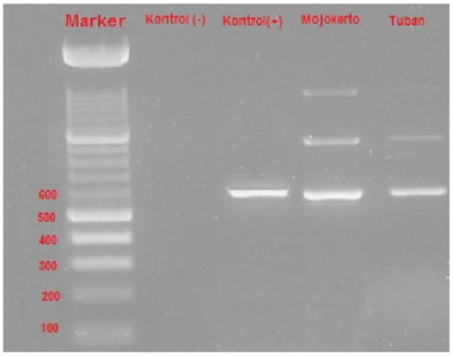


**Figure 2.** Gene analysis coding enterotoxigenic *E. coli* DNA with PCR electrophoresis was visualized on 2% agarose gel ethidium bromide staining. The results show a 600 bp cDNA string on all samples from chicken farms in Mojokerto and Tuban.

Enterotoxigenic *E. coli* DNA detection by PCR was showed a positive reaction for all isolates tested the PCR fragments which was located at position 600 bp. The used of primer has a great prospect when used in the early detection of enterotoxigenic *E. coli* strain enterohemorrhagic for local (Indonesian) because it has a fairly high specificity.[2]

Furthermore, enterotoxigenic *E. coli* resistance to some antibiotics such as amoxicillin, ampicillin, erythromycin, cephalosporins, tetracycline, cloxacilin and gentamicin. The using of antibiotics is widely used in the livestock

industry to prevent infection of *E. coli*.[22] because *E. coli* is a commensal bacterium that has a live strain not only in the gastrointestinal tract but also in various internal organs.[23] Bacteria become resistant to antibiotic agents due to mutations, transformations, transduction or conjugation.[24] The mechanism of resistance can be through various means, among others: drug activation, altering the structure of enzymes or bacterial membranes, decreasing the accumulation of drugs by cells, the presence of variations in metabolic pathways as well as increased metabolic concentration.[25]

Enterotoxigenic *E. coli* resistance to some antibiotics is showed resistant to amoxicillin, amphicillin, erythromycin, cephalosporins, tetracycline, cloxacilin and gentamicin. The ability of meniran (*Phyllanthus niruri* Linn) extract in inhibiting the growth of bacteria due to the chemicals contains found in plant meniran (*Phyllanthus niruri* Linn) extracts namely flavonoids, tannins and saponins.

Plant phenolic compounds and phenol compounds in general is a class of materials that has the ability to kill and inhibit the growth of bacteria. Damage to one of the constituent structures of bacterial cells can cause changes in the structure and working of bacteria.[22]

After meniran extract already for application to broiler chicken, further observations were made on the activation test from the meniran extract against enterotoxigenic. The meniran extract potential for enterotoxigenic *E. coli* was showed the power to kill the enterotoxigenic *E. coli* at concentrations of 30% (Table 1).

**Table 1.** Mean and Standard Deviation (SD) Number of *E. coli* (MPN) in Broiler Chickens with Various Treatment

| Treatments | Mean ± SD |
|---|---|
| T0 - (control group, not infected) | $0.7593^{a} \pm 0{,}32578$ |
| **T3 (infected by enterotoxigenic *E. coli* + 30% extract meniran)** | $1.1799^{a} \pm 0{,}.81489$ |
| **T2 (infected by enterotoxigenic *E. coli* + 25% extract meniran)** | $1.4210^{ab} \pm 0{,}32792$ |
| **T1 (infected by enterotoxigenic *coli* + 20% meniran extract)** | $2.3181^{bc} \pm 0{,}83574$ |
| **T0 + (group of chickens were infected by enterotoxigenic *E. coli*)** | $2.7732^{c} \pm 0.53645$ |

[a, b] Superscript different in the same column indicate significant differences ($p < 0.05$)

The analysis of data that has been done using Anova showed significant result between T0 + and T0-. While T0 + and T1 showed insignificant results, significant results were also seen between T0 + and T2. T0 + and T3 were significantly different, significant results were also seen between T0- and T1, and T1 and T3. Between T0- and T2 are not significant. While in T0- and T3 the results are significant. The concentration of 30% is a concentration that can kill the bacteria *E. coli* according to the result.

The cytoplasmic membrane is the outermost part cytoplasm that lies beneath the cell wall, composed of protein compounds, lipids and carbohydrates. This membrane acts to regulate the materials out of cells such as water and mineral salts needed cells. Parts of the cell in the cytoplasm include the ribosomes, nuclei, granules and mesosom. Ribosomes shaped small particles consisting of protein and ribonucleic acid (RNA), which functions as a protein synthesis. The tannin compound which is a component of meniran has a working mechanism that inhibits and kills the growth of bacteria by reacting with membrane cells as well as the destruction or inactivation of the function of the genetic material.[16] Tanin is also toxic and the nature of astrigensia works against bacterial cell membranes, by inhibiting certain enzymes.[24,25]

Other antibacterial compounds are saponins. Saponins can increase the permeability of bacterial cell membranes in order to alter membrane structure and function, causing membrane protein denaturation so that cell membranes will be damaged and lysis.[16] Saponins can increase the permeability of the intestinal wall, improve nutrient absorption and also inhibit the activity of the urease enzyme.[17]

Meniran plants also contain alkaloids that are toxic to microbes, so effectively kill gram-negative and gram-positive bacteria. Alkaloids act as antibacterial by destroying the peptidoglycan component in bacterial cells, so that the cell wall layer is not fully formed and causes the cell death.[26]

## CONCLUSION

*Enterotoxigenic E. coli* DNA was showed a positive reaction for all isolates tested the PCR fragments which was is located at position 600 bp. The enterotoxigenic *E. coli* are resistance to some antibiotics, such as amoxicillin, ampicillin, erythromycin, cephalosporins, tetracycline, cloxacillin and gentamicin. 30% concentration *Phyllanthus niruri* Linn extract effective as an antibacterial for the prevention of antibiotic resistance from enterotoxigenic *E. coli* of broilers.

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